Experimental and Applied Acarology (2005) 37: 183–198
DOI 10.1007/s10493-005-3260-9

Mode of inheritance of amitraz resistance in a Brazilian strain of the southern cattle tick, *Boophilus microplus* (Acari: Ixodidae)★

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Received 16 June 2005; accepted in revised form 13 September 2005

Key words: Amitraz resistance, *Boophilus microplus*, Cattle tick, Genetics, Inheritance

Abstract. The southern cattle tick, *Boophilus microplus* (Canestrini), has developed resistance to amitraz in several countries in recent years. A study was conducted at the USDA Cattle Fever Tick Research Laboratory in Texas to investigate the mode of inheritance of amitraz resistance with cross-mating experiments. The Muñoz strain, a laboratory reared acaricide-susceptible reference strain, was used as the susceptible parent and the Santa Luiza strain, originating in Brazil, was used as the resistant parent. A modified Food and Agriculture Organization Larval Packet Test was used to measure the levels of susceptibility of larvae of the parental strains, F_1, backcross, F_2, and F_3 generations. Results of reciprocal crossing experiments suggested that amitraz resistance was inherited as an incomplete recessive trait. There was a strong maternal effect on larval progeny's susceptibility to amitraz in both the F_1 and the subsequent generations. The values of the degree of dominance were estimated at -0.156 and -0.500 for the F_1 larvae with resistant and susceptible female parents, respectively. Results of bioassays on larval progeny of the F_1 backcrossed with the resistant parent strain and that of the F_2 generations suggested that more than one gene was responsible for amitraz resistance in the Santa Luiza strain. Comparisons of biological parameters (engorged female weight, egg mass weight, and female-to-egg weight conversion efficiency index) indicated significant differences between different genotypes. The differences appeared to be heritable, but not related to amitraz resistance. Results from this study may have significant implications for the management of amitraz resistance.

Introduction

The southern cattle tick, *Boophilus microplus* (Canestrini), is an important ectoparasite of cattle and a major vector of bovine babesiosis that causes severe economic losses to the cattle industry in many tropical and sub-

★This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation by the USDA for its use.

tropical regions of the world (Bram et al. 2002). Chemical acaricides have been used extensively for several decades to control this important pest. The intensive use of acaricides has led to the development of resistance to almost all major classes of acaricides in many countries, including Australia, Mexico, and Brazil. In Mexico, *B. microplus* first developed resistance to organophosphate (OP) acaricides in the 1980s (Aguirre et al. 1986). Pyrethroid acaricides were introduced to control OP-resistant ticks in the late 1980s, and resistance to pyrethroid acaricides emerged in the early 1990s (Fragoso et al. 1995). Many of the tick populations subsequently became resistant to both OP and pyrethroid acaricides (Santamaría et al. 1999). Amitraz was introduced in Mexico for the control of *B. microplus* at the same time as pyrethroids in the mid-1980s, but its use was limited during the early years. Amitraz became increasingly important in controlling ticks that were resistant to both the OP and pyrethroid acaricides in the late 1990s. The first case of amitraz resistance in Mexico was detected in 2001 in the state of Tabasco (Soberanes et al. 2002), and many more amitraz-resistant tick populations were found in several other states (Rodriguez-Vivas 2003; Li et al. 2004).

Amitraz has been used for the control of cattle ticks for over 40 years in various parts of the world. The development of amitraz resistance in *B. microplus* has been slow compared to other acaricides. In Australia, amitraz resistance was first detected in 1980 (Nolan 1981), but resistance was confined to some localized areas despite increased amitraz use since that time (Kunz and Kemp 1994; Kemp et al. 2003). Amitraz resistance in *B. microplus* has also been reported only recently in South Africa, Colombia, and Brazil (Furlong 1999; Strydom and Peter 1999; Benavides et al. 2000; Miller et al. 2002). The emergence of resistance to amitraz and other acaricides in Mexican strains of *B. microplus* is a major concern to the USDA Cattle Fever Tick Eradication Program (CFTEP). Other chemical acaricides have been used by CFTEP to eradicate outbreaks of cattle fever ticks from the quarantine zone along the US-Mexican border (George 1996; Li et al. 2004), and amitraz has the potential to be used to eradicate OP- and/or pyrethroid-resistant ticks. Although amitraz resistance can now be detected with more than one bioassay technique, the mechanisms of amitraz resistance and the factors affecting the evolution of amitraz resistance are not well understood. Successful management of pesticide resistance requires thorough understanding of the genetic, biological, and operational factors that influence the evolution of pesticide resistance in pest populations (Georghiou and Taylor 1986). The genetic components of resistance include the number and initial frequency of resistance alleles, dominance of resistance alleles, intensity of selection, and relative fitness of genotypes (Georghiou and Taylor 1986).

We have previously measured and characterized amitraz resistance in a Brazilian strain (Santa Luiza strain) and several Mexican strains of *B. microplus* (Li et al. 2004). The Santa Luiza strain demonstrated up to 154-fold resistance to amitraz, the highest level of resistance among all *B. microplus*

strains so far studied with a modified Food and Agriculture Organization (FAO) bioassay technique (Miller et al. 2002). Thus, this tick strain offered a unique opportunity to elucidate the mechanisms of resistance to amitraz, as well as to study the genetic basis of amitraz resistance in *B. microplus*. The objective of this study was to determine the mode of inheritance of amitraz resistance in *B. microplus* via cross-mating experiments.

Materials and methods

Tick strains

Two strains of *B. microplus* were used in this study. The Santa Luiza strain is an amitraz-resistant tick strain collected from a ranch in Brazil, and was maintained at the Mexican National Parasitology Laboratory, Jiutepec, Morelos, Mexico before being established at the USDA Cattle Fever Tick Research Laboratory (CFTRL) in Mission, Texas in 2000. The Muñoz strain is a susceptible laboratory strain that was established at the CFTRL in 1999 from an outbreak of *B. microplus* ticks in Zapata County, Texas. The Muñoz strain was susceptible to all major classes of acaricides, therefore, was used as the susceptible parental strain to cross with the resistant Santa Luiza strain in this study.

Host animals

A total of 12 Hereford heifer calves that were approximately 6–9 months of age and weighed approximately 250 kg were used in this study. The individually-tagged calves had no prior exposure to *Boophilus* ticks and were randomly assigned to be infested with one of the tick strains or genotypes at a particular time throughout the course of this study. The heifers were individually stanchioned in a covered, open-sided barn with walls separating each calf to prevent engorged ticks from escaping. A heifer was used only once and removed from the stanchion after all female ticks that had reached repletion were collected.

Parental strains

Two heifers were first infested with 0.5 g (ca. 10,000 individuals) of larvae each that were 21 days old from the Muñoz and Santa Luiza strains, respectively, with the vials containing the larvae glued to the back of each animal. Approximately 250 metanymphs were removed from each host at 13–14 days post infestation. The metanymphs of each strain were placed collectively, in separate 25×95 mm (8-dram) shell vials stopped with a cotton plug, and left in an incubator at 30 ± 2 °C and 92.5% RH to allow molting to adults. The

adults were separated by sex within 24 h of moulting, and were used in reciprocal crosses. The ticks left on the animals were allowed to develop to repletion. Engorged females were collected and placed in individual vials in a separate incubator at 30 ± 2 °C and 92.5% RH. Biological data, such as individual female weight, egg mass weight, and hatching rate were collected. Larvae hatched from mixed eggs were used for bioassays when they reach 14–16 days old.

Reciprocal crosses

Four orthopedic stockinette sleeves were glued to the side of each of two heifers for reciprocal crosses between adults of the resistant (Santa Luiza) and susceptible (Muñoz) tick strains. Eighty pair of Muñoz males and Santa Luiza females (type-I cross) were placed on one heifer with four sleeves, each containing 20 mating pair. The second heifer was infested with 80 pair of Santa Luiza males and Muñoz females (type-II cross) with each sleeve containing 20 mating pair. The engorged females were collected from each crossing type, and individual females were weighed and placed in individual vials in an incubator. After each female completed oviposition (20 d), the females were discarded and their egg masses were weighed. One fourth of the egg mass from each female was added to a vial of mixed eggs of the same crossing type and the resulting F_1 larvae were used for bioassays.

Backcrosses

Two heifers were each infested with 0.25 g (ca. 5000 individuals) mixed F_1 larvae from one of the two crossing types. A third heifer was infested with 0.25 g of Santa Luiza strain larvae. Approximately 250 metanymphs were removed from the heifer infested with one of the two F_1 larval types, and approximately 500 metanymphs were removed from the heifer infested with the Santa Luiza strain larvae. The metanymphs of each type were collectively placed in separate 25×95 mm (8-drum) shell vials in an incubator to allow molting to adults. Results of bioassays with F_1 progeny indicated that resistance was incomplete recessive and that the F_1 larvae from the type-II cross were more susceptible than that of the type-I cross. Therefore, only the F_1 type-II and the Santa Luiza strain were used for reciprocal backcrosses. Two additional heifers were infested each with one of the backcrossing types in four sleeves, each containing 20 pair of males and females, on the sides of the animals. The engorged females from each of the backcrossing types were collected, weighed, and placed in individual vials. Individual egg mass and hatching data were collected. Larvae from mixed eggs of each backcrossing type were obtained for bioassays as described above.

The genotypes of the parental strains, the Muñoz and Santa Luiza strains, and the F_1s from two different reciprocal crosses between the parental strains were designated as SS, RR, SR, and RS, respectively.

F_2 and F_3 generations

The metanymphs of both F_1 types that were left on the heifers (see above) were allowed to inbreed and to complete development on two separate heifers. The engorged females were collected and weighed, and the egg mass weight and the estimated hatching rate were recorded. The F_2 larvae from mixed eggs oviposited by engorged females of each of the F_1 crosses were tested for susceptibility to amitraz as described above. Similarly, the larvae of the F_2 type-II cross were reared to the F_3 generation and tested for susceptibility to amitraz.

Acaricide

The formulated amitraz (Taktic®, 12.5% EC) used in this study was a product of NOR-AM Chemical Company (Wilmington, DE).

Toxicity bioassay

A modified FAO Larval Packet Test (LPT), reported previously by Miller et al. (2002) and adopted by Li et al. (2004), was used for all amitraz bioassays in this study. Briefly, a top concentration (2% a.i.) of amitraz was prepared by adding a volume of the formulated amitraz to a mixture of trichloroethylene (Sigma, St. Louis, MO) and olive oil (Sigma) diluent with a final 2:1 ratio. Serial dilutions from the top dose were made using a diluent made of 2:1 trichloroethylene and olive oil. Nine and 16 amitraz concentrations, including the control (diluent only), were used for bioassays of larvae of the parent strains and progeny of the F_1, backcross, F_2 and F_3 generations, respectively. Each concentration had three replicates. A volume of 0.7 ml of each dilution was applied to a piece (7.5×8.5 cm) of nylon fabric (Type 2320, Cerex Advanced Fabrics, Pensacola, FL). The treated fabrics were placed on a hanging rack in a fume hood for 2 h to allow trichloroethylene to evaporate. The fabrics were then folded in half and sealed with bulldog clips on both sides forming a pocket. Fourteen- to 16-day-old larvae were used in bioassays. Approximately 100 larvae were placed into each packet with a fine brush, and the top was sealed with a third bulldog clip. The packets were placed in an incubator at 27 ± 2 °C, 90% RH for 24 h. The larval mortality in each packet was determined by counting the live and dead larvae in the packet.

Data analysis

The concentration-mortality responses of all amitraz bioassays were analyzed using the POLO-PC program (LeOra Software 1987). Mortality data of all three replicates of each concentration were included in probit analysis. Resistance Factors (RF) were calculated by dividing the LC_{50} of the Santa Luiza strain, F_1, backcrosses, F_2, or F_3 with the LC_{50} of the reference Muñoz strain. Differences between LC_{50} estimates were designated as significant when their 95% confidence intervals (CI) did not overlap.

The degree of dominance (D) of the resistance trait in the F_1 larvae from both reciprocal crosses was estimated using the formula $D = (2X_2–X_1–X_3)/(X_1–X_3)$, where X_1 is the log of the LC_{50} of the resistant strain, X_2 is the log of the LC_{50} of the F_1 and X_3 is the log of the LC_{50} of the susceptible strain (Falconer 1960; Stone 1962). The expected mortality at each amitraz concentration for larvae resulted from reciprocal backcrosses between F_1 type-II and the resistant strain (Santa Luiza), estimated on the basis of a single major gene, was calculated with the formula $X = (0.5)W_{(F_1)} + (0.5)W_{(R\ strain)}$, where X is the expected larval mortality at the given concentration and W is the mortality derived from their respective response lines of the parental types at the given concentration (Stone 1962, 1984). The expected mortality of the F_2 generation of both type-I and type-II crosses at each amitraz concentration was estimated using the formula $X = (0.25)W_{(S\ strain)} + (0.25)W_{(F_1\ type\text{-}I)} + (0.25)W_{(F_1\ type\text{-}II)} + (0.25)W_{(R\ strain)}$. The relationships between the observed and expected mortality in both backcross and F_2 generations were analyzed by χ^2 goodness-of-fit analysis, using procedures described by Tabashnik (1991) and Tapia-Perez et al. (2003), respectively.

Results

The genotypes and concentration-mortality responses of larvae from the susceptible and resistant parent strains, the F_1 from reciprocal crosses (type-I and type-II) between the parental strains, reciprocal backcrosses between the F_1 type-II and the resistant Santa Luiza strain, the F_2 and F_3 inbred generations of the type-II cross, and the F_2 inbred of the type-I cross are summarized in Table 1. The LC_{50} values of the susceptible parental strain (Muñoz) and the resistant parental strain (Santa Luiza) were measured at 0.0024 and 0.4519%, respectively. In comparison to the Muñoz strain, the Santa Luiza strain had a resistance factor of 188.3 to amitraz, with a relatively steep slope indicating a homogenous resistant strain.

The LC_{50} of F_1 larvae from the reciprocal cross type-I and type-II were 0.0219 and 0.0089%, with a resistance factor of 9.1 and 3.7, respectively, which were significantly higher than that of the susceptible Muñoz strain and lower than that of the resistant strain. Both LC_{50} values of the F_1 generations were closer to that of the Muñoz strain than to the Santa Luiza strain (Table 1,

Table 1. Summary of amitraz concentration–mortality responses of the parental strains, F_1, backcross, F_2 and F_3 generations of *B. microplus*.

Tick strain/crossing type	Genotypes of larval progeny[a]	Bioassay results				
		n	Slope (SE)	χ^2 (df)	LC_{50} (95% CI[b])	RF[c]
Muñoz	*SS*	2005	1.57 (0.07)	144.25 (19)	0.0024 (0.0015–0.0036)	1
Santa Luiza	*RR*	1001	4.60 (0.40)	50.62 (22)	0.4519 (0.3928–0.5076)	188
F_1 type-I	*SR*	2821	1.48 (0.05)	116.47 (28)	0.0219 (0.0174–0.0274)	9.1
F_1 type-II	*RS*	2584	1.96 (0.11)	123.27 (31)	0.0089 (0.0063–0.0117)	3.7
Backcross type-II (A)	*SR*, *RR*	3474	1.85 (0.09)	230.65 (43)	0.1258 (0.0902–0.1622)	52.4
Backcross type-II (B)	*RS*, *RR*	2731	0.99 (0.04)	241.29 (40)	0.0130 (0.0076–0.0204)	5.4
F_2 type-I	*SS*, *SR*, *RS*, *RR*	3941	1.89 (0.08)	165.77 (36)	0.0615 (0.0474–0.0768)	25.6
F_2 type-II	*SS*, *SR*, *RS*, *RR*	3515	1.49 (0.07)	149.96 (34)	0.0219 (0.0151–0.0293)	9.1
F_3 type-II	*SS*, *SR*, *RS*, *RR*	4243	1.82 (0.07)	94.37 (37)	0.0222 (0.0189–0.0257)	9.3

[a]Genotype designation: SS = susceptible homozygote, RR = resistant homozygote, SR = heterozygote with susceptible male and resistant female parents, RS = heterozygote with resistant male and susceptible female parents.
[b]CI = confidence interval.
[c]RF = resistance factor.

Figure 1). The value of the degree of dominance was −0.156 and −0.500 for the F_1 larvae from type-I and type-II crosses, respectively, suggesting that resistance to amitraz was inherited as an incomplete recessive trait.

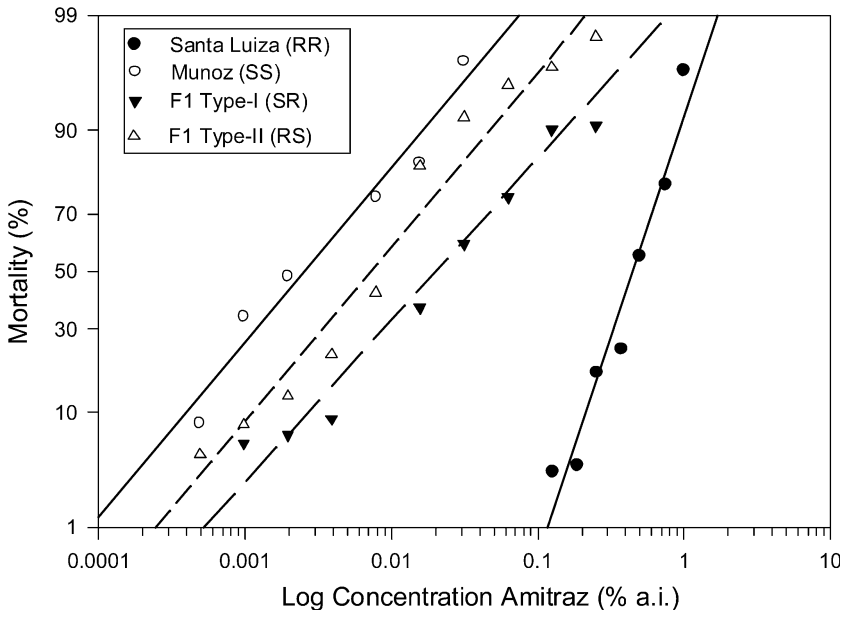

Figure 1. Amitraz concentration–mortality responses of the parental (Muñoz and Santa Luiza) strains and two types of F_1 generation resulting from reciprocal crosses between the parental strains.

The difference between LC_{50} values of F_1 larvae from the reciprocal crosses (type-I, and type-II) indicated a significant maternal effect on amitraz susceptibility of F_1 larvae. The F_1 larvae from the type-I cross, which had a resistant female parent (Santa Luiza), had a significantly higher LC_{50} estimate than that of the larvae from the type-II cross with a susceptible female parent (Muñoz) (Table 1, Figure 1).

Figure 2 illustrates the observed mortalities of larvae from reciprocal backcrosses between males and females of the F_1 type-II and the resistant parent (Santa Luiza strain) and the expected mortalities, which were calculated assuming monogenic inheritance. The type-A cross resulted from mating between males of the F_1 type-II and females of the Santa Luiza strain, and the type-B resulted from mating between males of the Santa Luiza strain and females of the F_1 type-II. Significant difference was detected between the observed and expected mortalities obtained in larval progeny from both type-A ($\chi^2 = 1734.8$, df $= 13$, $p = 0.000$) and type-B ($\chi^2 = 1228.9$, df $= 13$, $p = 0.000$; Figure 2a, b) backcrosses. The results suggest that more than one gene is involved in amitraz resistance in the Santa Luiza strain of *B. microplus*. The LC_{50} of the larval progeny from the backcross (RR × RS) that involved a resistant male parent and a heterozygous female parent was significantly lower than that of the larval progeny from its reciprocal type of the backcross (RS × RR) (Table 1), again suggesting a strong maternal effect on larval progeny susceptibility to amitraz.

Significant differences were also detected between the observed and expected mortalities of larvae of both F_2 type-I ($\chi^2 = 378.1$, df $= 10$, $p = 0.000$) and F_2 type-II ($\chi^2 = 223.9$, df $= 10$, $p = 0.000$) generations (Figure 3a, b). These results provided additional evidence that further support the conclusion of the involvement of multiple genes in amitraz resistance as demonstrated by results of the backcross experiments. The larvae of both types of the F_2 generation (type-I and type-II) had identical genotype compositions, consisting of equal proportions of SS, SR, RS, and RR. However, the LC_{50} of the F_2 type-1 was significantly higher than that of the F_2 type-II, concurring with previous observations that indicated a strong maternal effect on amitraz susceptibility in progeny, as shown in larval progeny from the F_1 and backcross generations (Table 1).

The LC_{50} of the F_3 type-II was not statistically different from that of the F_2 type-II. The genotypes of larvae in both generations were identical, consisting of equal proportions of RR, RS, SR, and SS. The results indicate the stability of amitraz resistance in successive generations of the same breeding line.

Table 2 summarizes the results of three biological parameters in the parental strains, the F_1, backcrosses, and F_2 generations. As shown in both the parental strains and the F_1 generation, the RR genotype of Santa Luiza strain had a significantly higher mean for the engorged female weight, egg mass weight, and CEI than the SS genotype of the Muñoz strain. Results of backcrosses indicated that both the SR and RS heterozygous females produced significantly more eggs with a higher CEI when mated with homozygous resistant (RR)

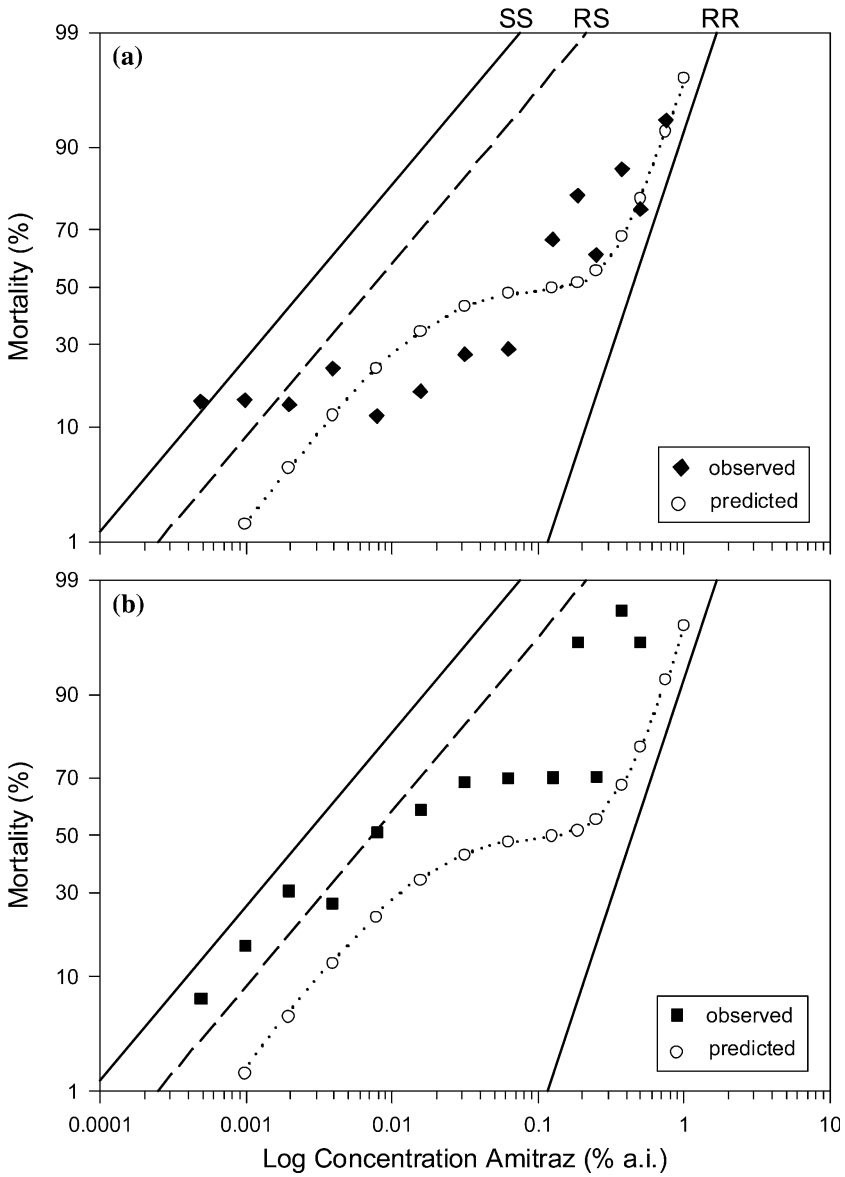

Figure 2. Comparisons between the observed and predicted mortalities in larval progenies of reciprocal backcrosses between the F_1 type-II and the resistant parent strain (Santa Luiza). (a) Backcross between the F_1 type-II males and the resistant parent (Santa Luiza) females. (b) Backcross between the resistant parent (Santa Luiza) females and the F_1 type-II males.

males than the reciprocal crosses. While the mean weight of the SR females was not significantly different from that of the RR females in the type-I backcross, the mean weight of the RS females was significantly different from the RR

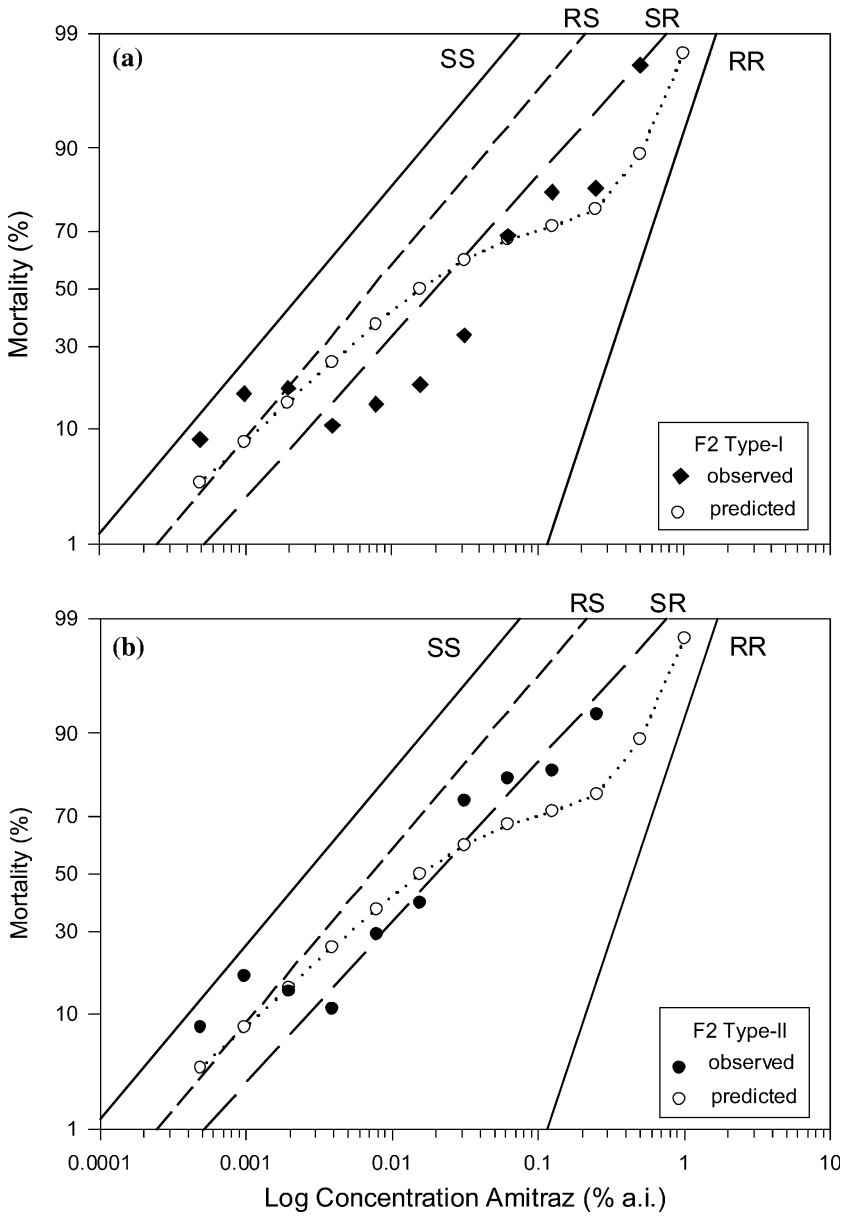

Figure 3. Comparisons between the observed and predicted mortalities in the larval progenies of F_2 generation, resulting from inbreeding of F_1 type-I (a) and F_1 type-II (b).

females in the type-II backcrosses. Although the CEI was significantly higher in F_2 type-I than in F_2 type-II, no significant differences were found between SR and RS females in engorged female weight or egg mass weight.

Table 2. Summary of biological parameters in the parental strains, the F_1, backcrosses, and F_2 generations.

Strain or Cross type	Parent genotype		Larval progeny genotypes	Engorged female weight (g)		Egg mass weight (g)		CEI[c] (%)	
	Male	female		*n*	Mean[b] (SD)	*n*	Mean[b] (SD)	*n*	Mean[b] (SD)
Muñoz	SS	**SS**	SS	50	0.349 (0.058)	49	0.129 (0.051)	49	37.4 (14.8)
Santa Luiza	RR	**RR**	RR	80	0.372 (0.060)*	80	0.194 (0.052)**	80	52.2 (11.3)**
F_1 type-I	SS	**RR**	SR	80	0.389 (0.052)**	76	0.218 (0.036)**	76	56.2 (7.2)**
F_1 type-II	RR	**SS**	RS	80	0.344 (0.065)	77	0.124 (0.056)	77	36.6 (15.4)
Backcross type-I (A)[a]	SR	**RR**	SR, RR	68	0.350 (0.055)	68	0.190 (0.041)	68	54.8 (11.0)
Backcross type-I (B)[a]	RR	**SR**	RS, RR	72	0.359 (0.055)	72	0.216 (0.037)**	72	60.1 (6.2)**
Backcross type-II (A)	RS	**RR**	SR, RR	68	0.337 (0.049)	68	0.197 (0.037)	68	58.5 (7.0)
Backcross type-II (B)	RR	**RS**	RS, RR	70	0.372 (0.048)**	69	0.228 (0.039)**	69	61.3 (8.6)*
F_2 from F_1 type-I	SR	**SR**	SS, SR, RS, RR	50	0.317 (0.059)	49	0.162 (0.043)	49	51.4 (10.3)*
F_2 from F_1 type-II	RS	**RS**	SS, SR, RS, RR	50	0.315 (0.062)	48	0.143 (0.058)	48	44.9 (14.4)

[a]Only biological data were collected. No bioassays were conducted on backcross type-I.
[b]ANOVA: * = significant ($p < 0.05$), ** highly significant ($p < 0.0001$).
[c]CEI, Conversion Efficiency Index = (egg mass weight/engorged female weight) × 100.

Discussion

The modes of inheritance of resistance to various pesticides have been studied in many pest species, including insects (Payne et al. 1988; Heim et al. 1992; Huang et al. 1999; Daborn et al. 2000; Bouvier et al. 2001), spider mites (Rizzieri et al. 1988; Goka 1998; Uesugi et al. 2002), and ticks (Lourens 1979, 1980). The modes of resistance to organochlorine and organophosphate acaricides have been well documented in *B. microplus* from early studies in Australia (Stone 1962; Wilson et al. 1971; Stone et al. 1973; Stone and Youlton 1982). Resistance to these compounds was found to be conferred by a single gene (dieldrin, dimethoate) or more closely related genes (diazinon, chlorpyrifos). These resistant genes were found to be autosomal with incomplete dominance in these studies. Similarly, resistance to organochlorine was found to be inherited as a single, near-complete dominant gene in two other tick species, *Amblyomma variegatum* (F.) and *Rhipicephalus appendiculatus* Neumann (Lourens 1979, 1980). A recent study on flumethrin resistance in a Mexican strain of *B. microplus* demonstrated that resistance to flumethrin was controlled by more than one gene, and expressed as a recessive or dominant trait depending on the flumethrin concentration exposed (Tapia-Perez et al. 2003). The results of our current study revealed a different mode of inheritance for amitraz resistance in *B. microplus*.

Resistance to amitraz in the Santa Luiza strain of *B. microplus* was inherited as an incomplete recessive trait involving more than one gene, and there was a strong maternal effect on the expression of amitraz resistance in the larval progeny. The mode of action of amitraz is believed to be interference with nervous system function of the targeted pest species by binding to the octopamine receptors (Evans and Gee 1980). Several different types of octopamine receptors have been identified in insects (Blenau and Baumann 2001), and a putative octopamine-like, G-protein-coupled receptor has also been reported in *B. microplus* (Baxter and Barker 1999). Although there was evidence suggesting the involvement of metabolic detoxification mechanisms in amitraz resistance, mutation of the octopamine receptors was speculated to be the main mechanism of resistance to amitraz (Li et al. 2004). Given the possibility of amitraz resistance involving both target site and metabolic resistance mechanisms, it is not surprising to find the polygenic nature of amitraz resistance in *B. microplus*. The involvement of multiple genes and the maternal effect on the resistance level to diflubenzuron were similarly demonstrated in a laboratory-selected strain of *Lucilia cuprina* (Wiedemann) (Kotze and Sales 2001). Although sex-linked inheritance of pesticide resistance has been demonstrated in several insect species (Daly and Fisk 1998; de Lame et al. 2001; Shearer and Usmani 2001), we were unable to test sex-related response in *B. microplus* because we used a modified FAO larval bioassay technique to test larvae for which males and females are indistinguishable. The mode of resistance to amitraz in nymphs and adult ticks may not necessarily be the same as in the larvae. It has been demonstrated in *Helicoverpa armigera* that resistance to

endosulphan was partially dominant in larvae but semi-recessive in adults (Daly and Fisk 1998). Compared with autosomally inherited traits, the sex-linked resistance may enhance or retard the rate of evolution of resistance (McDonald and Schmidt 1990; Daly and Fisk 1998).

Tapia-Perez et al. (2003) observed reduced egg mass weight in a pyrethroid-resistant strain of *B. microplus*, which was suggested to be a resistance-related fitness cost. Within our laboratory conditions, the Santa Luiza strain had a higher reproductive capacity than the Muñoz strain, as demonstrated by the measured biological parameters (Table 2). The discrepancies observed between the Santa Luiza and Muñoz strains were likely due to disparate geographic origins of these strains, instead of a resistance-related fitness difference.

One of the major benefits of understanding the mode of inheritance of resistance to a particular pesticide is that it helps to predict the evolution of resistance in the field, as well as to develop sound resistance management strategies using the information generated from such studies. The field populations of *B. microplus* in Mexico were shown to have low order (~5X) resistance to amitraz (Li et al. 2004), suggesting that they were likely to have susceptible homozygote (SS) and heterozygote (RS/RS) genotypes with few or no resistant homozygotes (RR). In the state of Yucatan in Mexico, ticks resistant to amitraz were found on about 19.4% of the ranches surveyed (Rodriguez-Vivas 2003). Since the heterozygotes (RS/SR) are relatively susceptible to amitraz compared with the resistant homozygotes (RR), use of a relatively higher concentration of amitraz that can kill both susceptible homozygotes and mildly resistant heterozygotes would allow elimination of resistant alleles from the population, consequently reducing the chance of formation of the homozygous resistant genotype (RR) and reducing the rate of resistance development. However, such a strategy may not work once the frequency of resistant homozygotes (RR) in a population becomes sufficiently high. The high concentration strategy proposed here is based on bioassay data collected using larvae in laboratory. It may not necessarily apply to field conditions where ticks of different developmental stages coexist and acaricides are applied with different methods. A different amitraz resistance management strategy of using a lower amitraz concentration was proposed in Australia (Kemp et al. 2003). In their study, the percentage survival of heterozygotes was intermediate between homozygous susceptible and homozygous resistant ticks. Based on efficacy trials on cattle of ticks of different genotypes, they determined that amitraz resistance was semi-dominant. It has been argued that, at a lower amitraz concentration, heterozygotes would not have more of a selective advantage, as more susceptibles would also have survived. The difference between our data and theirs may be caused by the different experimental approaches used or the resistant tick strain they used may have a resistance mechanism and/or mode of inheritance different from the Santa Luiza strain we studied. Their observations may have been suitably vague because they were unable to determine accurately the dominance of the heterozygotes with the tests available at that time. We are able to provide a more accurate

estimation of the degree of dominance in both heterozygotes with the modified larval packet technique in laboratory. Nevertheless, further study of the resistant tick strains with varying levels of amitraz resistance under field trial conditions would help clarify the issues.

Acknowledgements

We thank Dave Krska and Michael Moss for their excellent technical assistance, Homer Vasquez, Ruben Ramiraz, and James Hellums for maintaining tick strains and handling animals. We also thank Drs. Andy Chen, Jian-Zhou Zhao for reviewing this manuscript.

References

Aguirre J., Sobrino A., Santamaría M., Aburto A., Roman S., Hernandez M., Ortiz M. and Ortiz Y.A. 1986. Resistancia de garrapatas en Mexico. In: Cavazzani A.H. and Garcia Z. (eds), Seminario Internacional de Parasitologia Animal. Cuernavaca, Morelos, Mexico, pp. 282–306.

Baxter G.D. and Barker S.C. 1999. Isolation of a cDNA for an octopamine-like, G-protein coupled receptor from the cattle tick, *Boophilus microplus*. Insect Biochem. Mol. Biol. 29: 461–467.

Benavides E., Rodríguez S.C. and Romero A. 2000. Isolation and partial characterization of the Montecitos strain of *Boophilus microplus* (Canestrini) multi-resistant to different acaricides. Ann. NY Acad. Sci. 916: 668–671.

Blenau W. and Baumann A. 2001. Molecular and pharmacological properties of insect biogenic amine receptors: Lessons from *Drosophila melanogaster* and *Apis mellifera*. Arch. Insect Biochem. Physiol. 48: 13–38.

Bram R.A., George J.E., Reichard R.E. and Tabachnick W.J. 2002. The threat of foreign arthropod-borne pathogens to livestock in the United States. J. Med. Entomol. 39: 405–416.

Bouvier J.C., Bues R., Boivin T., Boudinhon L., Beslay D. and Sauphanor B. 2001. Deltamethrin resistance in the codling moth (Lepidoptera: Tortricidae): inheritance and number of genes involved. Heredity 87: 456–462.

Daborn P.J., McKenzie J.A. and Batterham P. 2000. A genetic analysis of cyromazine resistance in *Drosophila melanogaster* (Diptera: Drosophilidae). J. Econ. Entomol. 93: 911–919.

Daly J.C. and Fisk J.H. 1998. Sex-linked inheritance of endosulphan resistance in *Helicoverpa armigera*. Heredity 81: 55–62.

de Lame F.M., Hong J.J., Shearer P.W. and Brattsten L.B. 2001. Sex-related differences in the tolerance of Oriental fruit moth (*Grapholita molesta*) to organophosphate insecticides. Pest Manag. Sci. 57: 827–832.

Evans P.D. and Gee J.D. 1980. Action of formamidine pesticides on octopamine receptors. Nature 281: 60–62.

Falconer D.S. 1960. Introduction to Quantitative Genetics. Ronald, New York, 365 pp.

Fragoso H., Soberanes N., Ortiz M., Santamaría M. and Ortiz A. 1995. Epidemiologia de la resistencia a ixodicidas piretroides en garrapatas *Boophilus microplus* en la Republica Mexicana. In: Rodriqurz S. and Fragoso H. (eds), Seminario internacional de parasitologia animal-Resistncia y Control en Garrapatas y Moscas de Importancia Veterinaria. Acapulco, Guerrero, Mexico, pp. 45–57.

Furlong J. 1999. Diagnosis of the susceptibility of the cattle tick, *Boophilus microplus*, to acaricides in Minas Gerais state, Brazil. In: Fragoso H.S. and García Z.V. (eds), IV Seminario internacional de parasitologia animal- Control de la resistancia en garrapatas y moscas de importancia

veterinaria y enfermededades que transmiten. CONASAG-INIFAP-INFARVET-IICA-AM-PAVE-FILASA. Puerto Vallarta, Jal. México, pp. 41–46.

Georghiou G.P. and Taylor C.E. 1986. Factors influencing the evolution of resistance. In: National Research Council (eds), Pesticide Resistance-Strategies and Tactics for Management. National Academy Press, Washington, DC, pp. 157–169.

George J.E. 1996. The campaign to keep *Boophilus* ticks out of the United States: technical problems and solutions. In: Proceedings of the 100th Annual Meeting of the U.S. Animal Health Association. Spectrum, Richmond, VA, pp. 196–206.

Goka K. 1998. Mode of inheritance of resistance to three new acaricides in the Kanzawa spider mite, *Tetranychus kanzawai* Kishida (Acari: Tetranychidae). Exp. Appl. Acarol. 22: 699–708.

Heim D.C., Kennedy G.G., Gould F.L. and Van Duyn J.W. 1992. Inheritance of fenvalerate and carbofuran resistance in Colorado beetles- *Leptinotarsa decemlineata* (Say)- from North Carolina. Pestic. Sci. 34: 303–311.

Huang F., Buschman L.L., Higgins R.A. and McGaughey W.H. 1999. Inheritance of resistance to *Bacillus thuringiensis* toxin (Dipel ES) in the European corn borer. Science 284: 965–967.

Kemp D.H., Holdsworth P.A. and Green P.E. 2003. Registration of products for *Boophilus* control: suggestions for change from experience in Australia. In: García Z.V. and Fragoso H.S. (eds), V International Animal Parasitology: World Situation of Parasite Resistance in Veterinary Medicine. October 1–3, 2003, SENASICA-INIFAP-INFARVET-USDY-FAO-AMPAVE. Merida, Yucatan, México, pp. 1–11.

Kotze A.C. and Sales N. 2001. Inheritance of diflubenzuron resistance and monooxygenase activities in a laboratory-selected strain of *Lucilia cuprina* (Diptera: Calliphoridae). J. Econ. Entomol. 94: 1243–1248.

Kunz S.E. and Kemp D.H. 1994. Insecticides and acaricides: resistance and environmental impacts. Rev. Sci. Tech. Off. Ini. Epiz. 13: 1249–1286.

Li A.Y., Davey R.B., Miller R.J. and George J.E. 2004. Detection and characterization of amitraz resistance in the southern cattle tick *Boophilus microplus* (Acari: Ixodidae). J. Med. Entomol. 41: 193–200.

LeOra Software 1987. A user's Guide to Probit or Logit Analysis. LeOra Software, Berkeley, CA.

Lourens J.H. 1979. Genetic basis for organochlorine resistance in *Amblyomma variegatum* and information on the susceptibility on *A. lepidum* to organochlorine acaricides. J. Econ. Entomol. 72: 790–793.

Lourens J.H. 1980. Inheritance of organochlorine resistance in the cattle tick *Rhipicephalus appendiculatus* Neumann (Acari: Ixodidae) in East Africa. Bull. Ent. Res. 70: 1–10.

McDonald P.T. and Schmidt C.D. 1990. Linkage, expression, and distribution of the pyrethroid resistance gene in the horn fly (Diptera: Muscidae). J. Econ. Entomol. 83: 1718–1722.

Miller R.J., Davey R.B. and George J.E. 2002. Modification of the food and agriculture organization larval packet test to measure amitraz-susceptibility against Ixodidae. J. Med. Entomol. 39: 645–651.

Nolan J. 1981. Current developments in resistance to amidine and pyrethroid tickicides in Australia. In: Whitehead G.B. and Gibson J.D. (eds), Proceedings of Tick Biology and Control Conference. Tick research Unit, Rhodes University, Grahamstown, South Africa, pp. 109–114.

Payne G.T., Blenk R.G. and Brown T.M. 1988. Inheritance of permethrin resistance in the tobacco budworm (Lepidoptera: Noctuidae). Entomol. J. Econ. 81: 65–73.

Rizzieri D.A., Dennehy T.J. and Glover T.J. 1988. Genetic Analysis of Dicofol Resistance in Two Populations of Twospotted Spider Mite (Acari: Tetranychidae) from New York Apple Orchards. J. Econ. Entomol. 81: 1271–1276.

Rodriguez-Vivas I. 2003. Prevalence and potential risk factors for amitraz resistance in *Boophilus microplus* ticks in cattle from the state of Yucatan, Mexico. In: García Z.V. and Fragoso H.S. (eds), V International Animal Parasitology: World Situation of Parasite Resistance in Veterinary Medicine. October 1–3, 2003, SENASICA-INIFAP-INFARVET-USDY-FAO-AMPAVE, Merida, Yucatan, México, pp. 32–36.

Santamaría V.M., Soberanes C.N., Ortiz N.A., Fragoso S.H., Osorio M.J., Martínez I.F., Franco B.L., Delabra V.G., Quezada D.R., Giles H.I. and Ortiz E.M. 1999. Analisis de la situacion Actual ediante el monitoreo de susceptibilidad a ixodicidas en *Boophilus microplus* de 1993 a 1999 y medidas preventivas para retardar la resistencia al amitraz en Mexico. In: IV Seminario Internacional de Parasitologia Animal: Control de la Resistancia en Garrapatas y Moscas de Importancia Veterinaria y Enfermedades que transmiten, 20–22 de Octubre 1999. Puerto Vallarta, Jalisco, Mexico, pp. 103–117.

Shearer P.W. and Usmani K.A. 2001. Sex-related response to organophosphorus and carbamate insecticides in adult Oriental fruit moth, *Grapholita molesta*. Pest Manag. Sci. 57: 822–826.

Soberanes N.C., Santamaría M.V., Fragoso H.S. and García Z.V. 2002. First case reported of amitraz resistance in the cattle tick *Boophilus microplus* in Mexico. Téc. Pecu. Méx. 40: 81–92.

Stone B.F. 1984. The genetics of resistance. In: Griffiths D.A. and Bowman C.E. (eds), Acarology VI, Vol. 1. Ellis Horwood Limited, Chichester, England, pp. 441–448.

Stone B.F. 1962. The inheritance of Dieldrin resistance in the cattle tick, *Boophilus microplus*. Aust. J. Agric. Res. 13: 1008–1022.

Stone B.F., Wilson J.T. and Youlton N.J. 1973. Inheritance of dimethoate resistance in the Mackay strain of the cattle tick (*Boophilus microplus*) in Australia. Aust. J. Biol. Sci. 26: 445–451.

Stone B.F. and Youlton N.J. 1982. Inheritance of resistance to chlorpyrifos in the Mt Alford strain and to diazinon in the Gracemere strain of the cattle tick (*Boophilus microplus*). Aust. J. Biol. Sci. 35: 427–440.

Strydom T. and Peter R. 1999. Acaricides and *Boophilus* spp. resistance in South Africa. In: Fragoso H.S. and García Z.V. (eds), IV Seminario internacional de parasitologia animal-Control de la resistancia en garrapatas y moscas de importancia veterinaria y enfermededades que transmiten. CONASAG-INIFAP-INFARVET-IICA-AMPAVE-FILASA, Puerto Vallarta, Jal. México, pp. 35–40.

Tabashnik B.E. 1991. Determining the mode of inheritance of pesticide resistance with backcross experiments. J. Econ. Entomol. 84: 703–712.

Tapia-Perez G., García-Vazquez Z., Montaldo H. and George J.E. 2003. Inheritance of resistance to flumethrin in the Mexican Aldama strain of the cattle tick *Boophilus microplus* (Acari: Ixodidae). Exp. Appl. Acar. 31: 135–149.

Uesugi R., Goka K. and Osakabe M. 2002. Genetic basis of resistances to chlorfenapyr and etoxazole in the two-spotted spider mite (Acari: Tetranychidae). J. Econ. Entomol. 95: 1267–1274.

Wilson J.T., Stone B.F. and Wharton R.H. 1971. Inheritance of diazinon resistance in the Biarra strain of the cattle tick (*Boophilus microplus*) in Australia. Aust. J. Agric. Res. 22: 169–175.